DATA EVALUATION RECORD

8/2/2000

ZIRAM

2-1

Study Type: 84-2; Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test)

Work Assignment No. 2-15A (MRID 00147462)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Review Section IV, Toxicology Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Salmonella typhimurium/mammalian activation gene mutation assay
OPPTS Number: 870.5265 OPP Guideline Number: §84-2

DP BARCODE: D214220 SUBMISSION CODE: S485268
P.C. CODE: 034805 TOX. CHEM. NO.: 931

TEST MATERIAL (PURITY): FMC 13581 Technical (93.6% ai)

SYNONYMS: Zinc dimethyldithiocarbamate

CITATION: Wojciechowski, J.P., and Cascieri, Jr., T. (1984) Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test), FMC Corporation, Princeton, New Jersey. Lab Study Number A84-1317 July 12, 1984. MRID 00147462. Unpublished.

SPONSOR: FMC Corporation, 2000 Market Street, Philadelphia, PA

EXECUTIVE SUMMARY:

In a reverse gene mutation assay in bacteria (MRID 00147462), strains TA98, TA100, TA1535, TA1537 and TA1538 of Salmonella typhimurium were exposed to FMC 13581 technical (93.6% ai) in dimethylsulfoxide in the presence and absence of S9 mammalian metabolic activation. The five S. typhimurium strains were evaluated with FMC 13581 technical at concentrations of 10, 33.3, 66.6, 100, and 333.3 µg/plate (+/-S9).

FMC 13581 (93.6% ai) was tested up to twice the limit dose (10,000 µg/plate). Cytotoxicity was observed at a concentration > 333.3 µg/plate. The positive controls induced the appropriate responses in the corresponding strains.

FMC 13581 induced a genotoxic response in the TA100 tester strain; there was a 2.1 fold increase in mutant colonies over background at 66.6 µg/plate with metabolic activation and a 2.3 fold increase at 333.3 µg/plate without metabolic activation. In the TA1535 strain with metabolic activation, there was a 2.2 and 2.8 fold increase at 66.6 µg/plate and 100 µg/plate, respectively, but these increases in mutant colonies in the TA1535 strain were not of sufficient magnitude to be evaluated as positive.

FMC 13581 is considered positive for inducing gene mutations in the Salmonella assay in strain TA100 with and without activation.

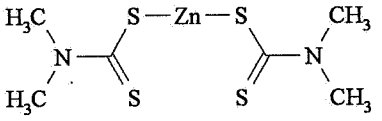
This study is classified as **acceptable**, and satisfies the requirements for FIFRA Test Guideline 84-2 for in vitro mutagenicity bacterial reverse gene mutation data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: FMC 13581, technical
Description: White powder
Lot/Batch #: E3459-127-1
Purity: 93.6% ai
Stability of compound: Reported as "not completely defined"
CAS #: 137-30-4
Structure:



Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:
Negative: DMSO
Solvent/final concentration: DMSO/50 µL per plate

Positive:
Nonactivation:

Sodium azide	5	µg/plate TA100, TA1535
2-Nitrofluorene	5	µg/plate TA98, TA1538
9-Aminoacridine	75	µg/plate TA1537
Other (list):		

Activation:
2-anthramine 4 µg/plate TA98, TA100, TA 1537, TA1535, and TA1538

3. Activation: S9 derived from

x Aroclor 1254	x induced	x rat	x liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other			___ other

The S9:cofactor mix was (1:9) and 0.5 mL of the S9 mix was used per plate. Ten mLs of the S9 mix contained:S9 (1 mL), 0.4M $MgCl_2$ (0.2 mL), 0.1M NADP (0.4 mL), 1.0M glucose-6-phosphate (0.05 mL), and 0.2M Na-phosphate buffer (5.0 mL). The S-9 mix was prepared immediately prior to use.

4. Test organisms: S. typhimurium strains
___ TA97 _x_ TA98 _x_ TA100 ___ TA102 ___ TA104
_x_ TA1535 _x_ TA1537 _x_ TA1538 ; list any others:

Properly maintained? **Yes**
Checked for appropriate genetic markers (rfa mutation, R factor)? **Yes**

5. Test compound concentrations used

Preliminary cytotoxicity test: Ten dose levels of Ziram technical (10, 33.3, 66.7, 100.0, 333.3, 666.7, 1,000.0, 3,333.3, 6,666.7, and 10,000.0 µg/plate) were evaluated with the S. typhimurium strain TA100 with and without S9 activation.

Mutagenicity assay: S. typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 were evaluated with Ziram technical at 10, 33.3, 66.6, 100, and 333.3 µg/plate with and without S9 activation.

B. TEST PERFORMANCE

1. Type of Salmonella assay:
_x_ standard plate test
___ pre-incubation (___ minutes)
___ "Prival" modification *(i.e. azo-reduction method)*
___ spot test
___ other *(describe)*

2. Protocol: The test substance was diluted in DMSO to specified concentrations; dilutions of the positive control substances were not described. For the activation conditions, 0.1 mL of the appropriate tester strain culture (density not reported), 0.5 mL of the S9 mix, and 0.05 mL of test material solution, solvent, or positive control were mixed with 2.0 mL of melted top agar supplemented with histidine and biotin. The mixture was poured over minimal agar (25 mL per plate). For nonactivation conditions, 0.5 mL of phosphate buffer, substituting for the S9 mix, was added to 2.5 ml of top agar. For the cytotoxicity test, single plates were prepared for each dose. For the mutagenicity assays, triplicate plates were prepared

for each dose, strain, and condition and incubated for 48-72 hours days at 37 ± 3 C. Plates were evaluated for toxicity, total revertant colonies per plate, and mean ± standard deviations for each dose point. Plates that were not counted immediately following incubation, were stored at 4 ± 2 C until colony counting could be conducted.

3. Evaluation Criteria

(a) Assay validity: If no clear positive response was observed after repeating an assay, "the initial positive test data (would) lose significance."

(b) Positive response: The test material was considered mutagenic if either (1) and/or (2) of the following conditions were met: (1) there was a dose-related increase in the number of revertants and the mean number of revertants per plate was ≥2x the vehicle controls in the TA98 and/or TA100 strains; (2) there was a dose-related increase in the number of revertants and the mean number of revertants per plate was ≥3x the vehicle controls for one or more of the TA1535, TA1537, and TA1538 bacterial strains.

II. REPORTED RESULTS

A. Analytical determinations: No data were presented indicating the actual concentrations of the test stock solutions.

B. Preliminary cytotoxicity assay: The number of colonies/plate for the preliminary assay are presented in Attachment 1 (study report Table 1, pages 16 and 17) of this DER. Ten dose levels (10-10,000 µg/plate) were evaluated with the S. typhimurium strain TA100 with and without S9 activation. Single plates were used per dose and per condition. A solvent control was run. Cytotoxicity apparent as inhibition of growth was observed in the cultures treated at ≥666.7 µg/plate.

Based on these results, all strains of S. typhimurium were assayed with the test substance at 10-333.3 µg/plate (+/-S9).

C. Mutagenicity assay: A summary of the mutagenicity assay is presented in Attachment 2 (study report page 20) of this DER. The test substance ranging from 10-333.3 µg/plate (±S9) was evaluated in triplicate plate cultures using strains TA98, TA100, TA1535, TA1537, and

TA1538. It was stated that bacterial background lawn evaluation codes were taken; the data however, were not submitted. Except for strain TA1538 without metabolic activation, the positive controls induced marked increases in revertant colonies in their respective strains. The sponsor reported that during the procedure, the positive control (2-nitrofluorene) may not have been added to the TA1538 strain resulting in an absence of response. There was evidence of induced mutant colonies over background in the TA100 tester strains. FMC 13581 induced a genotoxic response in the TA100 tester strain both with and without S9 metabolic activation. A dose response relationship was observed with a 2.1 fold increase in mutant colonies over background at 66.6 µg/plate with metabolic activation and a 2.3 fold increase at 333.3 µg/plate without metabolic activation. Revertants per plate were elevated for other strains, particularly the TA1535.

Based on these results, the study author concluded that FMC 13581 caused a positive response in tester strain TA100 in this microbial gene mutation assay.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The reviewer agrees with the study author's conclusion that FMC 13581 was mutagenic in tester strain TA100 under the conditions of this microbial gene mutation assay. The reviewer notes that in the TA1535 strain with metabolic activation, there was a 2.2 and 2.8 fold increase at 66.6 µg/plate and 100 µg/plate, respectively, but these increases in mutant colonies in the TA1535 strain were not of sufficient magnitude to be evaluated as positive.

FMC 13581 was assayed over an appropriate dose range as it was tested to 2x the limit concentration (10,000 µg/plate) with the S. typhimurium strains. The sensitivity of the test system to detect mutagenesis was adequately demonstrated by the response obtained with the nonactivated and S9-activated positive controls. The study is classified as acceptable.

FMC 13581 is considered positive for inducing gene mutations in the Salmonella assay in strain TA100 with and without activation.

B. Study deficiencies - The report indicated that bacterial background lawn evaluations were performed for the mutagenicity assay. The background lawn data were not submitted for the mutagenicity assay, but were included for the preliminary assay. This deficiency

does not alter the conclusions of this study.

SignOff Date:	8/2/00
DP Barcode:	D172447
HED DOC Number:	014277
Toxicology Branch:	RAB2